

TEST REPORT

INTERLABORATORY COMPARISON STUDY IX (2005) ON THE DETECTION OF *SALMONELLA* spp. ORGANISED BY CRL-*SALMONELLA*

Laboratory code	
Laboratory name	
Address	
Country	
Date of arrival of the parcels - - 2005
Start time of storage of the capsules at - 20 °C	Date:..... Time:.....
Start time of storage of the faeces and dust samples at + 5 °C	Date:..... Time:.....
Parcels damaged?	YES NO
Starting date testing - - 2005

Is your laboratory accredited or certified for the detection of <i>Salmonella</i> . If yes, according to which system ? If no, are you planning to be accredited or certified in the near future ?	
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PRE-ENRICHMENT – Buffered Peptone Water (BPW) (I)**Medium information BPW**

What did you use to prepare the BPW?

Individual ingredients

Dehydrated medium

Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of BPW

Name

Code number

Batch number

Expire date

Specific data of composition of BPW medium. What is the concentration of the following compounds in 1000 ml water:

Enzymatic digest of casein

Sodium chloride

Disodium hydrogen phosphate dodecahydrate

Potassium dihydrogen phosphate

Preparation of BPW

Date of preparation

..... - - 2005

pH after preparation

....., measured at °C

pH at the day of use

....., measured at °C

Did you perform quality control of BPW?

yes

no

PRE-ENRICHMENT – Buffered Peptone Water (BPW) (II)**Prewarming time and temperature of the BPW**

At the start	Date: - - 2005 time: h min temperature incubator: °C
At the end	Date: - - 2005 time: h min temperature incubator: °C

Incubation time and temperature for dissolving the capsules

At the start	Date: - - 2005 time: h min temperature incubator: °C
At the end	time: h min temperature incubator: °C

Incubation time and temperature for pre-enrichment: (4 ± ½) hrs

At the start	Date: - - 2005 time: h min temperature incubator: °C
At the end	Date: - - 2005 time: h min temperature incubator: °C

Incubation time and temperature for pre-enrichment: (18 ± 2) hrs

At the start	Date: - - 2005 time: h min temperature incubator: °C
At the end	Date: - - 2005 time: h min temperature incubator: °C

SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (I)

Medium information MSRV

What did you use to prepare the MSRV?

Individual ingredients

Dehydrated medium

Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of MSRV
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Name	
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Code number	
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Batch number	
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Expire date	
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Specific data of composition of MSRV medium. What is the concentration of the following compounds in 1000 ml water:
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Tryptose	
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Casein hydrolysate	
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Sodium chloride	
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Monopotassium dihydrogen phosphate	
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Magnesium chloride (anhydrous)	
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Malachite green oxalate	
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Agar	
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Novobiocin	
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Preparation of MSRV

Date of preparation - - 2005
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pH after preparation, measured at °C
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pH at the day of use, measured at °C
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Did you perform quality control of MSRV?	yes	no
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SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (II)
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Incubation time and temperature for selective enrichment after (4 ± ½) hrs incubation of BPW

At the start of the first period (first 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the end of the first period (first 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the start of the second period (second 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the end of the second period (second 24h)	Date: - - 2005 time: h min temperature incubator: °C

Incubation time and temperature for selective enrichment after (18 ± 2) hrs incubation of BPW
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At the start of the first period (first 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the end of the first period (first 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the start of the second period (second 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the end of the second period (second 24h)	Date: - - 2005 time: h min temperature incubator: °C

OWN SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (I)

If you use more selective media, please write these on an annex.

Medium:

Medium information

What did you use to prepare the medium?

Individual ingredients

Dehydrated medium

Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of the medium

Name

Code number

Batch number

Expire date

Specific data of composition of the medium. What is the concentration of the compounds in 1000 ml water:

Preparation of the medium

Date of preparation

..... - - 2005

pH after preparation

....., measured at °C

pH at the day of use

....., measured at °C

Did you perform quality control of the medium?

yes no

OWN SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (II)
Further details concerning the medium

Volume of the medium per jar/tube in ml	
Inoculation volume of BPW	
Prescribed incubation temperature in °C	

Incubation of BPW

Did you perform the selective enrichment of your own medium after: (4 ± ½) h incubation of BPW ?	YES / NO
Did you perform the selective enrichment of your own medium after: (18 ± 2) h incubation of BPW ?	YES / NO

Incubation time and temperature for own selective enrichment medium

At the start of the first period (first 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the end of the first period (first 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the start of the second period (second 24h)	Date: - - 2005 time: h min temperature incubator: °C
At the end of the second period (second 24h)	Date: - - 2005 time: h min temperature incubator: °C

FIRST AND SECOND ISOLATION - Xylose Lysine Desoxycholate medium (XLD) (I)**Medium information XLD**

What did you use to prepare the XLD ?

Individual ingredients

Dehydrated medium

Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of XLD

Name

Code number

Batch number

Expire date

Specific data of composition of XLD medium. What is the concentration of the following compounds in 1000 ml water:

Xylose

L-lysine

Lactose

Sucrose

Sodium chloride

Yeast extract

Phenol red

Agar

Sodium desoxycholate

Sodium thiosulphate

Ferric ammonium citrate

Saccharose

Preparation of XLD

Date of preparation

..... - - 2005

pH after preparation

....., measured at °C

pH at the day of use

....., measured at °C

Did you perform quality control of XLD ?

yes

no

FIRST AND SECOND ISOLATION - Xylose Lysine Desoxycholate medium (XLD) (II)**Size of petri dishes**

Size of petri dishes used	90 mm	100 mm	140 mm
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Incubation time and temperature for isolation after (4 ± ½) hrs incubation of BPW

Start incubation of XLD, inoculated from 24h MSRV.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of XLD, inoculated from 24h MSRV.	Date: - - 2005 time: h min temperature incubator: °C
Start incubation of XLD, inoculated from 48h MSRV.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of XLD, inoculated from 48h MSRV.	Date: - - 2005 time: h min temperature incubator: °C

Incubation time and temperature for isolation after (18 ± 2) hrs incubation of BPW

Start incubation of XLD, inoculated from 24h MSRV.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of XLD, inoculated from 24h MSRV.	Date: - - 2005 time: h min temperature incubator: °C
Start incubation of XLD, inoculated from 48h MSRV.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of XLD, inoculated from 48h MSRV.	Date: - - 2005 time: h min temperature incubator: °C

FIRST AND SECOND ISOLATION – Second isolation medium for choice (I)

If you use more selective media, please write these on an annex.

Name of the medium	
Prescribed incubation temperature in °C	

Medium information of second isolation medium

What did you use to prepare the second isolation medium ?
Individual ingredients
Dehydrated medium
Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of second isolation medium

Name	
Code number	
Batch number	
Expire date	

Specific data of composition of second isolation medium. What is the concentration of the compounds in 1000 ml water:

Preparation of second isolation medium

Date of preparation - – 2005
pH after preparation, measured at °C
pH at the day of use, measured at °C
Did you perform quality control ?	yes no

FIRST AND SECOND ISOLATION - Second isolation medium for choice (II)**Size of petri dishes**

Size of petri dishes used	90 mm	100 mm	140 mm
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Incubation time and temperature for isolation after (4 ± ½) hrs incubation of BPW

Start incubation of second medium, inoculated from 24h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of second medium, inoculated from 24h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C
Start incubation of second medium, inoculated from 48h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of second medium, inoculated from 48h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C

Incubation time and temperature for isolation after (18 ± 2) hrs incubation of BPW

Start incubation of second medium, inoculated from 24h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of second medium, inoculated from 24h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C
Start incubation of second medium, inoculated from 48h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C
End incubation of second medium, inoculated from 48h MSR.V.	Date: - - 2005 time: h min temperature incubator: °C

FIRST AND SECOND ISOLATION – Own isolation medium (I)

If you use more selective media, please write these on an annex.

Name of the medium	
Prescribed incubation temperature in °C	

Medium information of own isolation medium

What did you use to prepare the second isolation medium ?
Individual ingredients
Dehydrated medium
Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of own isopation medium

Name	
Code number	
Batch number	
Expire date	

Specific data of composition of own isolation medium. What is the concentration of the compounds in 1000 ml water:

Preparation of own isolation medium

Date of preparation - – 2005
pH after preparation, measured at °C
pH at the day of use, measured at °C
Did you perfrom quality control ?	yes no

FIRST AND SECOND ISOLATION – Own isolation medium (II)**Size of petri dishes**

Size of petri dishes used	90 mm	100 mm	140 mm
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Incubation time and temperature for isolation after (4 ± ½) hrs incubation of BPW

Start inoculation of own isolation medium, inoculated from 24h MSRV.	Date: - – 2005 time: h min temperature incubator: °C
End inoculation of own isolation medium, inoculated from 24h MSRV.	Date: - – 2005 time: h min temperature incubator: °C
Start inoculation of own isolation medium, inoculated from 48h MSRV.	Date: - – 2005 time: h min temperature incubator: °C
End inoculation of own isolation medium, inoculated from 48h MSRV.	Date: - – 2005 time: h min temperature incubator: °C

Incubation time and temperature for isolation after (18 ± 2) hrs incubation of BPW

Start inoculation of own isolation medium, inoculated from 24h MSRV.	Date: - – 2005 time: h min temperature incubator: °C
End inoculation of own isolation medium, inoculated from 24h MSRV.	Date: - – 2005 time: h min temperature incubator: °C
Start inoculation of own isolation medium, inoculated from 48h MSRV.	Date: - – 2005 time: h min temperature incubator: °C
End inoculation of own isolation medium, inoculated from 48h MSRV.	Date: - – 2005 time: h min temperature incubator: °C

CONFIRMATION – Nutrient agar (II)

Preparation of the nutrient agar

Date of preparation - – 2005
pH after preparation, measured at °C
pH at the day of use, measured at °C
Did you perform quality control of nutrient agar ?	yes no

Size of petri dishes

Size of petri dishes used	90 mm	100 mm	140 mm
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BIOCHEMICAL CONFIRMATION

Manufacturer of the TSI agar

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2005

Manufacturer of the urea agar

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2005

Manufacturer of the l-Lysine decarboxylation medium

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2005

Manufacturer of other confirmation tests -

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2005

DETECTION BY PCR**General questions**

Is the PCR used commercially available	Yes No
If yes, name of PCR, manufacturer and batch used in the study:	
Is the PCR validated	Yes No
How much samples did you test for <i>Salmonella</i> using this PCR in 2004 ?	
At what moment did you start with the extraction/detection?	before or after incubation of BPW
Volume of pre-enrichment used for extraction	
Volume of DNA-sample obtained from extraction	
Volume of DNA-sample added to PCR-mixture	

Composition of PCR-mixture

Compound	Volume per sample	Manufacturer and batch of specific compound

Name of thermocycler	
Write down the cycles	
What kind of detection system is used ?	

Table 1: Results of isolation using MSR/V (dish numbers 1-25)
after (4 ± ½) hrs incubation of BPW

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium for choice		Own isolation medium		XLD		Second isolation medium for choice		Own isolation medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

a: Col = number of colonies used for confirmation; b:Sal= number of colonies confirmed as Salmonella

Table 1 (continued): Results of isolation using MSR/V (dish numbers D1-D10)
after (4 ± ½) hrs incubation of BPW

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium for choice		Own isolation medium		XLD		Second isolation medium for choice		Own isolation medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
D1												
D2												
D3												
D4												
D5												
D6												
D7												
D8												
D9												
D10												

a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as Salmonella

Table 1 (continued): Results of isolation using MSRV (dish numbers C1-C12)
after (4 ± ½) hrs incubation of BPW

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium for choice		Own isolation medium		XLD		Second isolation medium for choice		Own isolation medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												
C10												
C11												
C12												

a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as Salmonella

Table 2: Results of isolation using MSR V (dish numbers 1-25)
after (18 ± 2) hrs incubation of BPW

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium for choice		Own isolation medium		XLD		Second isolation medium for choice		Own isolation medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as Salmonella

Table 2 (continued): Results of isolation using MSR/V (dish numbers D1-D10)
after (18 ± 2) hrs incubation of BPW

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium for choice		Own isolation medium		XLD		Second isolation medium for choice		Own isolation medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
D1												
D2												
D3												
D4												
D5												
D6												
D7												
D8												
D9												
D10												

a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as Salmonella

Table 2 (continued): Results of isolation using MSRV (dish numbers C1-C12)
after (18 ± 2) hrs incubation of BPW

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium for choice		Own isolation medium		XLD		Second isolation medium for choice		Own isolation medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												
C10												
C11												
C12												

a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as Salmonella

Table 3: Results of isolation using own selective enrichment medium (dish numbers 1-25)

sample no.	Own selective enrichment 24 hours						Own selective enrichment 48 hours					
	*		*		*		*		*		*	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

*: fill in the isolation medium used, a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as Salmonella

Table 3 (continued): Results of isolation using own selective enrichment medium
(dish numbers D1-D10)

sample no.	Own selective enrichment 24 hours						Own selective enrichment 48 hours					
	*		*		*		*		*		*	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
D1												
D2												
D3												
D4												
D5												
D6												
D7												
D8												
D9												
D10												

*: fill in the isolation medium used, a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as *Salmonella*

Table 3 (continued): Results of isolation using own selective enrichment medium
(dish numbers C1-C12)

sample no.	Own selective enrichment 24 hours						Own selective enrichment 48 hours					
	*		*		*		*		*		*	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												
C10												
C11												
C12												

*: fill in the isolation medium used, a: Col = number of colonies used for confirmation, b: Sal = number of colonies confirmed as *Salmonella*

Table 4: Results of detection using PCR (dish numbers 1-25, D1-D10 and C1-C12)

sample no.	PCR + or -			
		no.		no.
1		D1		C1
2		D2		C2
3		D3		C3
4		D4		C4
5		D5		C5
6		D6		C6
7		D7		C7
8		D8		C8
9		D9		C9
10		D10		C10
11				C11
12				C12
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				

Comment(s) on operational details that might have influenced the test results:

Name of person (s) carrying out the ninth
bacteriological collaborative study (2005)

Date and signature

Name of person in charge

Date and signature

Please send the completed test report before 16 December by fax or email to the CRL-Salmonella. The original test reports should also be sent to the CRL. Use the address below:

Petra Berk
RIVM/MGB (Pb63)
CRL-Salmonella
P.O. Box 1
3720 BA Bilthoven
The Netherlands

..Tel.: +31-30-2742093
Fax: +31-30-2744434
Email: Petra.Berk@rivm.nl